Autonomic Function in Children with Type 1 Diabetes Mellitus

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We investigated autonomic function in 58 children and young adults with Type 1 diabetes mellitus (aged 7–22 years, duration from 3 to 18, 8.6 ± 3.4 years) and in 74 healthy controls (6–21 years) using power spectral analysis of blood pressure and heart rate in addition to conventional standard autonomic function tests: deep breathing, the Valsalva manoeuvre, and a standing test. None of the diabetic patients were symptomatic. Reproducibility of the tests was assessed by determining the coefficient of variation in 9 controls (7.8–37.7 %). Thirteen per cent of the subjects had difficulty in adequately performing the Valsalva manoeuvre. After adjustment for age, sex, body mass index, and respiratory frequency, results of the Valsalva manoeuvre and deep breathing were not different between patients and controls and there was no significant postural reduction in systolic blood pressure (≥ 20 mmHg) in the patients. Heart rate variation in the supine position during natural breathing was low in patients, although power spectral analysis of heart rate variation did not show a significant decrease in the power density in the high and the low frequency in patients compared to healthy controls. Beat-to-beat blood pressure fluctuation was significantly lower in patients and correlated with metabolic control (mean annual haemoglobin A_{1c}), but not with disease duration and was abnormal in 7 diabetic children (12 %). In contrast, tests of vagal activity were not impaired in the patients in this age range. We concluded that vagal involvement in Type 1 diabetic patients determined by spectral analysis of R–R intervals in addition to conventional tests is uncommon, but that beat-to-beat blood pressure variation was more likely to be affected. © 1998 John Wiley & Sons, Ltd.

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Introduction

Autonomic neuropathy is a recognized complication of diabetes mellitus (DM) and involvement of cardiovascular autonomic innervation is associated with increased mortality.[1] During the past three decades, a number of autonomic function tests have been used to measure cardiac autonomic innervation, including heart rate responses to standing, the Valsalva manoeuvre, deep breathing, and pharmacological stimulation.[2] Studies in children with Type 1 diabetes mellitus using these conventional tests report that autonomic involvement may occur early,[3–5] but this is controversial.[6] Moreover, the methodology of autonomic function tests, including their reproducibility and sensitivity, has not been established in the paediatric field.

Recent advances in computer technology have made it possible to evaluate sympathetic and vagal control of the heart using a root mean squared successive difference technique and spectral analysis of heart rate variation.[7] Ziegler *et al*. suggest that spectral analysis of heart rate variability should be added to the battery of standard autonomic function tests.[8] However, few data are available in children with Type 1 DM using power spectral analysis, and sample numbers have been small.[9,10]

The present study was designed to investigate auto-

Abbreviations: BRSI baroreflex sensitivity index; CCV coefficient of component variance; DB deep (timed) breathing; FABP finger arterial blood pressure; HF high frequency; ID initial drop (in blood pressure on standing); LF low frequency; OS overshoot (in blood pressure on standing); R Rwave (of ECG); UB uncontrolled (ie natural) breathing; VI valsalva index

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nomic function using a new computerized method of assessing beat-to-beat variation in blood pressure and heart rate, in addition to conventional autonomic tests in children with Type 1 DM and their healthy peers, to determine the reproducibility of these autonomic function tests in this population.

Subjects and Methods

Subjects

We examined 58 patients with Type 1 DM, 35 male, aged 7–22 years (mean age: 15.8 years) who were followed up in the Department of Paediatrics, Linkoping University Hospital. We also recruited 74 healthy controls, 38 male, aged 6–21 (mean age 14.5 years). Duration of diabetes ranged from 3 to 18 (8.6 ± 3.4) years. Metabolic control in patients was assessed by averaging annual HbA_{1c} from the onset of the disease. There were no significant differences in weight and height between patients and controls (Table 1). Informed consent was obtained for autonomic function tests from all control subjects and patients and their parents. The research protocol was approved by the Ethical Committee of Linkoping University Hospital.

Blood Pressure Measurement

Finger arterial blood pressure (FABP) was determined with the commercially available Finapres (Omeda, Model 2300) device.[11–13] Since FABP was found to be influenced by finger tip temperature,[12] we standardized finger temperature by dipping the subject's hands into hot water (40 °C) prior to BP measurement.[14] The hands were then covered with gloves to keep them warm. A finger cuff was attached to the middle phalanx of middle finger of the right hand. BP was measured at heart level in both the supine and upright positions. One of the investigators assisted subjects to keep the cuff at heart level during standing.

Table 1. Demography in Type 1 DM patients (DM) and healthy controls (N): systolic (SBP) and diastolic blood pressure (DBP)

	N or DM	Age (yr)	
		6–12	13–22
Sex (M/F)	N	18/9	20/27
	DM	6/5	29/18
Height (cm)	N	143 ± 13	172 ± 7
	DM	147 ± 13	171 ± 12
Weight (kg)	N	35 ± 9	64 ± 11
	DM	38 ± 10	66 ± 12
SBP/DBP	N	102 ± 10/55 ± 9	119 ± 14/59 ± 8
(mmHg)	DM	107 ± 9/57 ± 5	118 ± 15/63 ± 10[a]
Heart rate	N	75 ± 11	65 ± 8
	DM	79 ± 10	70 ± 12[a]
HbA_{1c} (%)		6.8 ± 0.6	7.2 ± 1.2
		(6.0–7.7)	(5–11.1)

Significant difference between the patients group and the controls, [a]$p < 0.05$.

Procedure

The test protocol was performed in a soundproof room, with temperature maintained between 22 and 23 °C. Food intake was not allowed for at least 2 h before the study and coffee was avoided for at least 12 h before investigation. FABP, three-lead ECG, and a respiratory curve by a nasal-tip thermistor were monitored and recorded by a Racal FM tape recorder. After a 7 min rest in the supine position, controlled deep breathing (inspiration for 4 s, expiration 4 s), a Valsalva manoeuvre and uncontrolled normal breathing (for 4 min) were performed sequentially with a 3 min interval between each test. Thereafter, subjects stood for 7 min. Patients were asked to stand quickly by themselves from supine without using their right hand. This was practised twice before the study and was usually completed within 3–4 s. When patients showed impending syncope with the symptoms of nausea, sweating and pallor, the test was immediately stopped and patients were returned to the supine position.

Data Collection and Analysis

FABP, ECG, and respiratory signals were A/D converted at a sampling frequency of 250 Hz and stored in a personal computer system (Hewlett Packard ES/12). An originally developed signal analysis program[15] was used to provide data on sequential R–R intervals of the ECG (ms), beat-to-beat systolic and diastolic FABP (mmHg), and respiratory phase during each manoeuvre. Parameters of autonomic function were determined as follows

Heart Rate Variation During Deep Breathing

We obtained sequential R–R intervals of the ECG during deep breathing (DB) and determined the coefficient of variation ($\%CVRR_{DB}$) and the difference in HR between the expiratory and the inspiratory phase (E – I difference).[2]

Valsalva Manoeuvre

The Valsalva manoeuvre was performed 3 min after a deep breathing test in the supine position in 101 subjects (50 controls and 51 patients). Subjects were instructed to blow into a mouthpiece, connected to a sphygmomanometer, and to maintain a pressure of 40 mmHg for 12 s. The manoeuvre was repeated twice if the BP and ECG recordings were inadequate. R–R intervals and FABP were monitored continuously. The Valsalva index (VI) was the ratio of the longest R–R interval during the release phase to the shortest R–R interval during the manoeuvre.[2] A baroreflex sensitivity index (BRSI) was obtained by the modified method of Palmero *et al.*:[17] BRSI = {the highest mean BP at overshoot (phase IV) – the lowest mean BP at release (phase III)}/the longest R–R interval at phase IV – the shortest R–R interval at phase III.

Beat-to-beat BP and Heart Rate Responses to Active Standing

Active standing has been reported to elicit a transient but a large fall in blood pressure (initial drop: ID) during the first 30 s (Figure 1), and this circulatory change is a good indicator for autonomic function.[14,16] We analysed heart rate, systolic and diastolic blood pressure at the following points according to our previous study:[14] supine (averaged for 30 s before standing), ID, overshoot (OS), 1, 2, 3, and 7 min after standing. FABP and heart rate at 1, 2, 3, and 7 min of standing were obtained from averaging consecutive beats over 10 s.

Heart Rate and BP Variation During Uncontrolled Breathing

Sequential R–R intervals and corresponding beat-to-beat systolic and diastolic FABP and respiratory phases during uncontrolled breathing were obtained in the supine position (for 4 min), during early standing (1–4 min) and late standing (4–7 min). Time series comprising 200–350 consecutive R–R intervals, systolic and diastolic FABP were manually checked by one of the investigators (HT) to exclude artifacts. Fluctuation of R–R intervals during uncontrolled breathing (UB) was assessed by a coefficient of variation ($\%CVRR_{UB}$: standard deviation/the mean R–R interval) and power spectral analysis. Power spectral density was computed with an original program for the autoregressive model.[17] The autoregressive coefficients were calculated using the Burg algorithm,[18] and the model order was chosen to minimize the Akaike's score. The program provided power spectral density and the peak frequency of R–R interval, systolic, diastolic FABP, and respiration, in addition to the integrals of the low (0.04–0.15 Hz) and the high frequency band (0.15–0.49 Hz). The coefficient of component variance was calculated for the low (CCVLF) and the high frequency band (CCVHF) of R–R intervals using the following formula:[19]

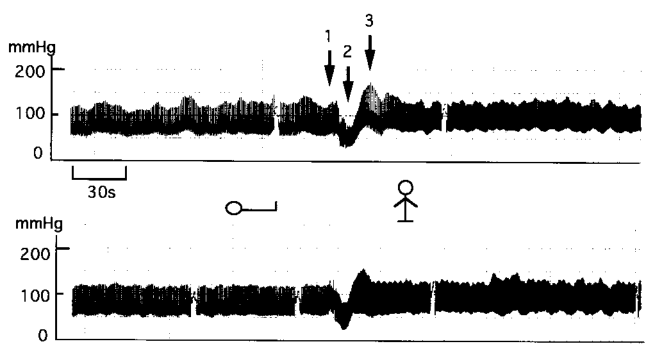

Figure 1. An original recording of beat-to-beat blood pressure and heart rate in a healthy control (upper panel) and in a patient with Type 1 DM (lower panel). The subject stood up at arrow 1. At the onset of standing a transient blood pressure drop (initial drop: ID, arrow 2) was followed by overshoot (OS, arrow 3). Note the decreased blood pressure (BP) and heart rate (HR) fluctuation in the patients in the supine position

CCVHF = (power spectral density of the high frequency band)$^{1/2}$/mean R–R interval $\times$ 100 (%) and CCVLF = (power spectral density of the low frequency band)$^{1/2}$/mean R–R interval $\times$ 100 (%).

CCVLF/CCVHF (LF/HF) was obtained for the evaluation of the sympathovagal balance of the heart. CCVLF, CCVHF and LF/HF were determined separately in the supine position ($CCVLF_{sup}$, $CCVHF_{sup}$ and LF/HF_{sup}) and during standing ($CCVLF_{st}$, $CCVHF_{st}$ and LF/HF_{st}).

BP fluctuation was evaluated by the coefficient of variation for systolic ($\%CV_{SBPsup}$) and diastolic ($\%CV_{DBPsup}$) in the supine position and during standing ($\%CV_{SBPst}$ and $\%CV_{DBPst}$). A component of coefficient variance of the high and the low frequency band was also calculated for systolic and for diastolic BP in the supine position and during standing ($CCVHF_{SBPsup}$, $CCVLF_{SBPsup}$, $CCVHF_{DBPsup}$, $CCVLF_{DBPsup}$, $CCVHF_{SBPst}$, $CCVLF_{SBPst}$, $CCVHF_{DBPst}$ and $CCVLF_{DBPst}$).

Statistics

To compare the variables of autonomic function mentioned above between the patient and the control group adjusting for age, gender, body dimension and respiration, multiple regression analysis was used including age, sex (male = 1, female = 0), body mass index (BMI), and respiratory frequency (Hz) in addition to the group code (Type 1 DM = 1, control = 0) as the independent variables. Thereafter, the standard regression coefficient (SC) was obtained to determine the association between each autonomic variable and its predictors. A p value of the standard coefficient of less than 0.05 was considered to indicate a significant association. To determine the reference data in healthy controls, the prediction value was calculated for each variable which showed an age-related change,[20] otherwise the confidence interval (mean $\pm$ 2 SD). The significance of differences was determined by Student's unpaired t-test. Chi-square analysis was used in the contingency table.

Results

Reliability of Autonomic Function Tests in Children

Reproducibility of autonomic function tests in 9 controls ranged from 7.7–37.7 % (Table 2). The power spectral analysis of R–R intervals did not provide as high a degree of reproducibility as $\%CVRR_{UB}$. All the subjects were able to perform deep breathing, while 13 % had difficulties in performing an adequate Valsalva manoeuvre.

Age-related Changes in Autonomic Function

Most of the parameters of autonomic function, including the coefficient of variation for deep breathing and E – I

Table 2. Within-subject variation (%) of each autonomic function test in 9 control subjects: the coefficient of variation (%CV_{DB}) and the difference in HR between the expiratory and inspiratory phase (E – I diff.) of R–R intervals during deep breathing, the Valsalva index (VI) during Valsalva manoeuvre, blood pressure changes to active standing at the initial drop (ID), overshoot (OS) and 7 min of standing

Deep breathing test	
%CV_{DB}	25.3 ± 15.1
E – I diff.	27.8 ± 9.0
Valsalva manoeuvre	
VI	17.9 ± 13.3
% Changes in SBP during standing	
OS	7.8 ± 5.3
7 min	20.0 ± 11.6
Variation of R–R interval in the supine position	
%$CVRR_{UB}$	37.6 ± 33.9
$CCVLF_{sup}$	30.8 ± 22.8
$CCVHF_{sup}$	28.6 ± 31.4
Variation of blood pressure supine position	
%CV_{SBPsup}	29.3 ± 20.0
%CV_{DBPsup}	18.8 ± 14.9
during standing	
%CV_{SBPst}	19.1 ± 10.5
%CV_{DBPst}	37.7 ± 21.3

Values are mean ± SD.

difference during deep breathing, BRSI by the Valsalva manoeuvre, coefficient of variation during uncontrolled breathing, $CCVHF_{sup}$ and $CCVLF_{sup}$ in the supine position, and FABP change at ID, showed significant age-related changes (Tables 3 and 4). Fluctuation of FABP during standing also did as shown in Table 4 and Figure 6. On the other hand, %CV_{SBP} and %CV_{DBP} in the supine position, systolic BP responses to standing at OS and after 7 min were little influenced by age.

Heart Rate Variation During Deep Breathing

All subjects successfully performed controlled deep breathing. Although there were no significant differences of the mean value of the coefficient of variation for deep breathing and E – I difference between controls and patients, one patient had a coefficient of variation for deep breathing (5.8 %) reduced below the normal limits (6.2–21.8 %).

Valsalva Manoeuvre

Several subjects (13% of those less than 13 years old and 6 % of those 13 years or more) who had difficulties in maintaining the expiratory phase at 40 mmHg were excluded from the analysis. According to Sharpey-Schafer[21] the arterial blood pressure response can be divided into two types; a square shaped and a sinusoid shaped. In control subjects of 13 years or more, 6 subjects showed a square shaped response and 31 a sinusoid shape, while in diabetic patients of 13 years or more, these shapes were 19 and 26, respectively ($p = 0.011$, X^2). The square shape was more frequently found in the patient group. One patient showed marked reduction in blood pressure (–80 mmHg for systolic BP from the basal level) during the phase II of the manoeuvre with transient loss of consciousness. There was no significant difference in the calculated VI and BRSI between the patient group and the control group and no patient had abnormal results.

Beat-to-beat BP and Heart Rate Responses to Active Standing

An original recording of blood pressure during standing in a control and diabetic subject is shown in Figure 1. The patient group had significantly higher heart rate and higher diastolic BP in the supine position than controls (Table 1). Heart rate was positively correlated to the average HbA_{1c} concentration ($r = 0.448$, $p < 0.01$). Systolic BP levels were not different between two groups.

BP and heart rate responses to standing at ID and 7 min showed significant age-related changes (Table 3). In controls 2 children showed near-fainting symptoms with reduction in FABP during standing, while none

Table 3. Relation of Type 1 DM, sex, age, and body mass index to each parameter of autonomic function: coefficient variation (%$CVRR_{DB}$) of R–R intervals and the difference in heart rate between the expiratory and inspiratory phase (E – I diff.) during deep breathing, the Valsalva index (VI) during the Valsalva manoeuvre, blood pressure changes to active standing at the initial drop (ID), overshoot (OS) and 7 min of standing

Variable	Deep breathing		Valsalva	Standing					
	%$CVRR_{DB}$	E – I diff.	VI $n = 86$	ID		OS		7 min	
				SBP	DBP	SBP	DBP	SBP	DBP
DM or control	0.041 (0.668)	0.097 (0.312)	–0.037 (0.745)	0.098 (0.266)	0.021 (0.808)	0.232 (0.015)	0.118 (0.226)	0.191 (0.044)	0.043 (0.647)
Age	–0.255 (0.053)	–0.266[a] (0.042)	0.107 (0.491)	–0.424[a] (0.0004)	–0.389[a] (0.0007)	0.051 (0.687)	0.127 (0.326)	0.218[a] (0.087)	0.336[a] (0.009)

Values are given as the standard coefficients determined by the multiple regression analysis and those of sex and body mass index are not shown because of no significant difference. p values given in parentheses. [a]$p < 0.05$.

Table 4. Relation of Type 1 DM, sex, age, body mass index (BMI) and respiration frequency (RF) to autonomic function evaluated (a) by the coefficient of variation of beat-to-beat systolic and diastolic blood pressure in the supine position ($\%CV_{SBPsup}$, $\%CV_{DBPsup}$) and during standing ($\%CV_{SBPst}$, $\%CV_{DBPst}$), and (b) by power spectral analysis in the low (CCVLF) and high frequency band (CCVHF) of sytolic (SBP) and diastolic (DBP) blood pressure

(a)

Variables	Supine		Standing	
	$\%CV_{SBPsup}$	$\%CV_{DBPsup}$	$\%CV_{SBPst}$	$\%CV_{DBPst}$
DM or control	–0.341[a] (0.002)	–0.447[a] (0.0001)	–0.188 (0.078)	–0.245[a] (0.027)
Age	–0.031 (0.844)	–0.213 (0.153)	0.337[a] (0.019)	0.408[a] (0.006)

(b)

	Supine				Standing			
	Systolic BP		Diastolic BP		Systolic BP		Diastolic BP	
	CCVLF	CCVHF	CCVLF	CCVHF	CCVLF	CCVHF	CCVLF	CCVHF
DM or control	–0.232[a] (0.016)	–0.100 (0.251)	–0.264[a] (0.004)	–0.078 (0.307)	–0.081 (0.467)	–0.062 (0.601)	–0.121 (0.273)	–0.145 (0.231)
Age	–0.109 (0.391)	–0.543[a] (0.0001)	–0.324[a] (0.008)	–0.609[a] (0.0001)	0.526[a] (0.0004)	0.015[a] (0.921)	0.527[a] (0.0004)	0.07 (0.651)

Values are given as the standard coefficients determined by the multiple regression analysis and those of sex and body mass index are not shown because of no significant difference. p values given in parentheses. [a]$p < 0.05$.

in the patient group had symptoms, or evidence of orthostatic hypotension.

Diabetic patients had significantly greater increases in systolic BP at OS and 7 min of standing than controls (Table 3) but without the difference in BP at ID.

Heart Rate and FABP Variation in the Supine Position

Respiratory frequency differed in different individuals during uncontrolled breathing. Therefore, prior to power spectral analysis of heart rate and FABP, we determined respiratory frequency using autoregressive analysis. When a single peak of the respiration power density was found in the range of 0.20 to 0.40 Hz (Figure 3), the data were considered suitable for power spectral analysis. One hundred and one children (80 %) showed a single peak of the respiratory frequency in the supine position and 97 children (76 %) during standing. Finally data from 83 children (65 %), 38 patients, and 45 controls, were used for multiple regression analysis, including respiratory frequency as a dependent variable.

Mean R–R intervals in the supine position were significantly lower in the patient group (Table 3) and decreased with increasing HbA_{1c} (SC: –0.496, $p = 0.007$), $CCVLF_{sup}$, $CCVHF_{sup}$, and $\%CVRR_{UB}$ of R–R intervals in the supine position showed age-related changes and the normal range expressed by the prediction interval is shown in Figures 2 and 4. In the supine position

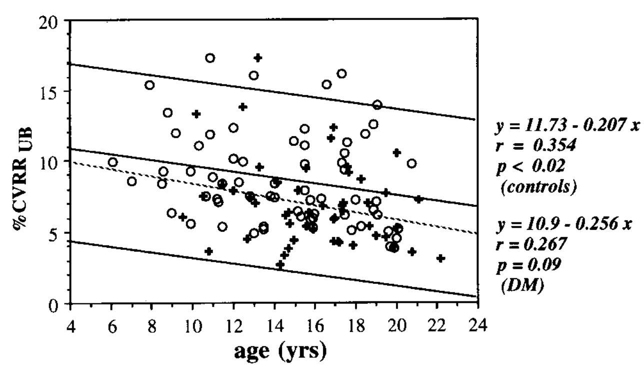

Figure 2. Plots of coefficient variation of R–R intervals ($\%CVRR_{UB}$) in relation to age in patients with Type 1 DM (+) and in healthy controls (o). The regression line of the patients (dashed line) and controls (straight line) with upper and lower limits of the normal range (prediction interval) are shown

$\%CVRR_{UB}$ was significantly lower in patients compared to controls (Table 5). Power spectral analysis indicated that both $CCVLF_{sup}$ and $CCVHF_{sup}$ tended to be lower in the patient group and that LF/HF did not differ between the two groups (Table 5). $CCVHF_{sup}$ was not related to HbA_{1c}.

BP fluctuation in the supine position ($\%CV_{SBP}$ and $\%CV_{DBP}$) was significantly lower in the patient group than in the control group (Table 4). $\%CV_{DBP}$ was negatively associated with HbA_{1c} (SC: –0.495, $p = 0.008$). Decreased BP fluctuation in patients was mainly due to the decreased power spectral density of the low frequency band,

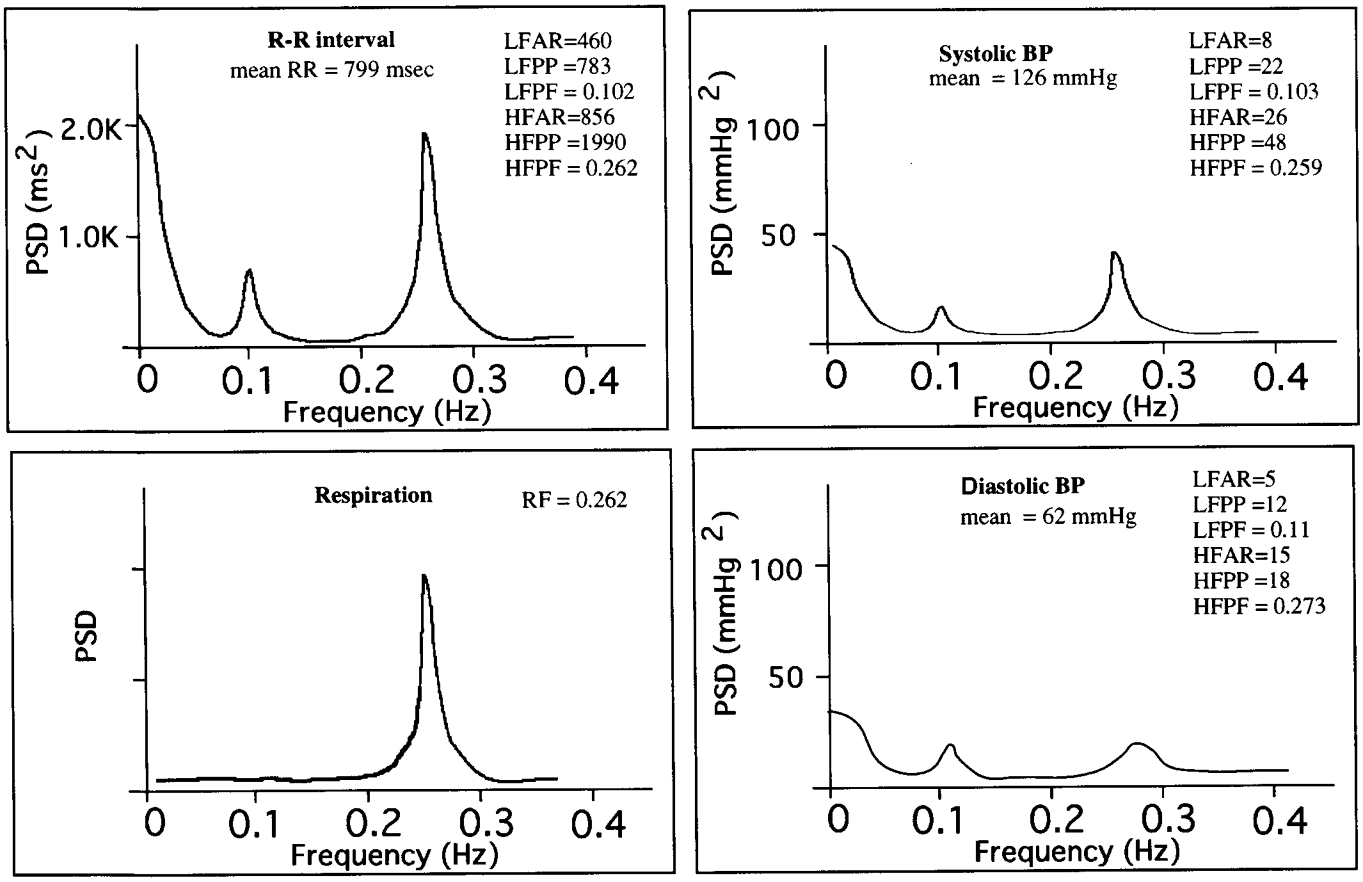

Figure 3. An original diagram of power spectral analysis of R–R intervals, systolic and diastolic finger arterial pressure, and respiration with quadruple windows in one subject. The integrated power of the low (LFAR) and high (HFAR) frequency band and the peak power of the low (LFPP) and high (HFPP) frequency band were automatically calculated with their peak frequency (LFPF, HFPF)

because the standard coefficient was statistically significant only in the low, but not in the high frequency band (see CCVLF of systolic and diastolic BP in the supine position, Table 4). Four patients showed abnormally low FABP fluctuation: in the supine position one in $\%CV_{SBP}$ and one in $\%CV_{DBP}$, during standing two in $\%CV_{SBP}$ and one in $\%CV_{DBP}$ (Figure 5).

Heart rate fluctuation during standing assessed by $\%CVRR_{UB}$, $CCVHF_{st}$ and $CCVLF_{st}$ did not differ between patients and controls, while BP fluctuation was significantly lower in patients than in controls (Table 4).

Duration and Autonomic Function

Duration of diabetes did not show a significant relation with parameters of autonomic function in this study when evaluated by multiple regression analysis (data not shown).

Discussion

Our data suggest that reproducibility of autonomic function tests in children is poor compared to that of adults decribed in previous studies.[8] The discrepancy may be explained by larger variation of autonomic activity in children than in adults.[15] Moreover, skill in performance of manoeuvres such as the Valsalva manoeuvre and metronome-controlled breathing may be less in children. Thirteen per cent of children in the present study could not perform an adequate Valsalva manoeuvre. Ringel *et al.* omitted the Valsalva manoeuvre from their study because of this.[3] Deep breathing with a metronome rhythm could be performed successfully for 1 min by young children, but could not easily be maintained for longer. The standing test was the easiest manoeuvre for children, and this and the deep breathing test had the best reproducibility.

Although power spectral analysis of R–R intervals has been successfully applied for the evaluation of cardiac autonomic innervation in adults,[22] there are few reports on their significance in children.[23,24] Moreover, methodology and reliability have not been established including influence of age, sex, body dimension, and respiration. To obtain good recordings of power density in the HF band of R–R intervals, natural and regular breathing is required with a single breath frequency. Because breath frequency influences cardiac vagal activity, it is essential to standardize breath frequency when comparing cardiac vagal tone in different subjects. Metronome-controlled breathing usually is employed in adults, but previous studies in children have paid little attention to breath frequency.[8,22,23] In this study vagal activity was assessed

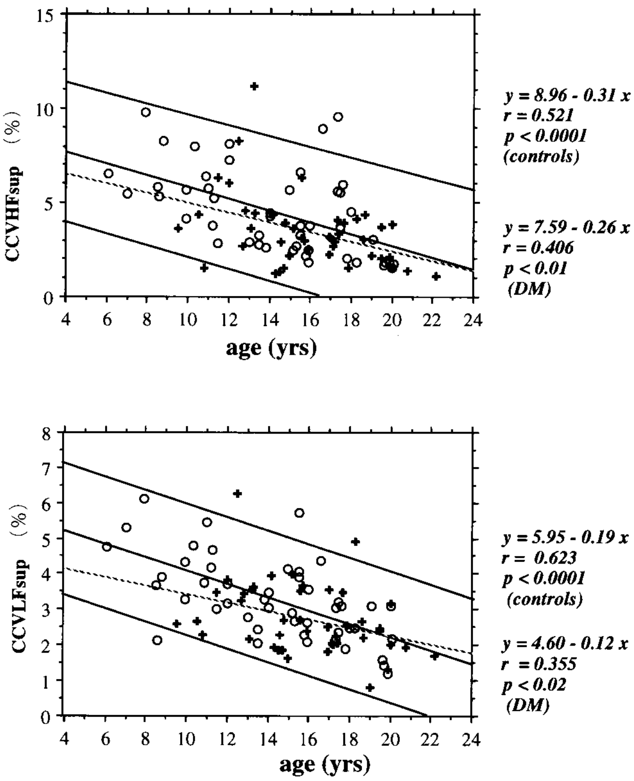

Figure 4. Plots of the coefficient of component variance in the low ($CCVLF_{sup}$, lower panel) and high frequency band ($CCVHF_{sup}$, upper panel) of R–R intervals in relation to age in patients with Type 1 DM (+) and in healthy controls (o). The regression line of the patient (dashed line) and control (straight line) with the upper and the lower normal limit determined by prediction interval are shown

during uncontrolled breathing, since some children had difficulty with metronome-controlled breathing over several minutes. As a result, 80 % of children had natural and relaxed breathing with a single respiratory frequency pattern in the supine position and 76 % of children during standing. $CCVHF_{st}$ and $\%CV_{SBPst}$ correlated with respiratory frequency. These findings indicate that uncontrolled breathing under relaxed conditions allows good recordings of R–R intervals with a single respiratory frequency in the majority of children.

Even so, power spectral analysis of heart rate variation did not give satisfactory results in terms of reproducibility. This may be due in part to the small number of subjects in this study, but we could not conclude that the analysis of the heart rate variation is a better method than conventional autonomic tests.

Most of the parameters of autonomic function showed significant age-related changes. An age-related range of healthy control values is always needed for the assessment of autonomic function in diabetes. In this study, we obtained age-related normal limits expressed by prediction value of CCVHF, -LF and $\%CVRR_{UB}$. These data can be utilized as reference in Caucasian children and young adults (Figures 2, 4 and 6).

Our patients with Type 1 DM did have evidence of reduced autonomic activity compared to non-diabetic peers. Heart rate fluctuation in the supine position ($\%CVRR_{UB}$) was significantly lower, although in power spectral analysis of R–R intervals the tendency for the power density of the high frequency band was not significant and heart rate variation during deep breathing expressed by $\%CV_{DB}$ and E – I difference did not differ. Overall, 7 diabetic children (12 %) had at least 1 abnormal result; 5 in blood pressure fluctuation, 1 in deep breathing, 1 in the Valsalva manoeuvre. These results reveal less frequent and low prevalence autonomic neuropathy in our patients compared to previous studies.[3–5]

Beat-to-beat FABP fluctuation in the supine position and during standing was lower in our patients than in controls. However, orthostatic hypotension was not found. FABP fluctuation appeared to be related to glycaemic control and was reduced with increasing HbA_{1c}. This is, to our knowledge, the first report that shows a reduction in beat-to-beat FABP fluctuation without severe cardiac vagal impairment.

Lower FABP fluctuation in diabetic patients might be explained by decreased sympathetic discharge or reduced postsynaptic responses to noradrenaline in the resistance vessels. The former alternative seems to be the most likely, since previous studies demonstrated that diabetic patients with autonomic neuropathy had higher BP responses to noradrenaline infusion due to denervation hypersensitivity.[25] Our patients had evidence of reduced rhythmic sympathetic outflow to the resistance vessels without damage of the cardiac vagal system. In keeping with this, Hoffman *et al*. demonstrated that Type 1 DM patients had lower muscle sympathetic activity than control subjects without apparent vagal involvement of the heart.[26] They concluded that the lower muscle sympathetic nervous activity is an early manifestation of diabetic autonomic neuropathy that precedes impaired cardiac parasympathetic control.

Whether diabetic autonomic neuropathy preferentially occurs in the vagal or sympathetic nervous system is controversial. Ewing *et al*.[7] suggested that cardiac parasympathetic fibres are involved more extensively and earlier than sympathetic nerves, based on the combined results of five conventional tests. However, recent studies using advanced computer technology have suggested that sympathetic involvement occurs early in Type 1 DM. Zgur *et al*.[27] reported early decrease of the sympathetic skin response amplitude in patients with moderate diabetic polyneuropathy and uptake of radioactive iodine-123-meta-iodo-benzylguanidine measured by single photon emission computed tomography (SPECT) is impaired more commonly than cardiac vagal control.[28] Our present findings are consistent with the hypothesis that cardiovascular sympathetic nerves are involved at least as early as cardiac parasympathetic fibres in diabetic neuropathy.

Heart rate fluctuation during deep breathing and BRI might not be a sensitive test in children. We did not find a difference between patients and controls. This is

Table 5. Relation of Type 1 DM, sex, age, respiration frequency (RF) and body mass index (BMI) to variation of R–R intervals during uncontrolled breathing (%$CVRR_{UB}$), mean R–R intervals, component coefficient of variation in the low and high frequency band of R–R intervals in the supine position and during standing ($CCVLF_{sup}$, $CCVHF_{sup}$, $CCVLF_{st}$, $CCVHF_{st}$, and CCVLF/CCVHF

Variables	%$CVRR_{UB}$		mean R–R interval	
	Supine	Standing (4–7 min)	Supine	Standing (4–7 min)
DM or control	–0.253[a] (0.018)	–0.137 (0.286)	–0.294[a] (0.007)	–0.166 (0.162)
age	–0.428[a] (0.006)	–0.009 (0.958)	0.340[a] (0.027)	0.110 (0.477)
RF	–0.084 (0.440)	–0.239 (0.056)	–0.064 (0.558)	–0.094 (0.420)

Variables	Supine			Standing (4–7 min)		
	$CCVLF_{sup}$	$CCVHF_{sup}$	LF/HF	$CCVLF_{st}$	$CCVHF_{st}$ n = 85	LF/HF
DM or control	–0.150 (0.122)	–0.156 (0.116)	0.044 (0.697)	–0.053 (0.684)	0.005 (0.963)	0.110 (0.300)
Age	–0.592[a] (0.0001)	–0.570[a] (0.001)	0.314 (0.538)	0.080 (0.634)	–0.475[a] (0.001)	0.695[a] (0.0001)
RF	–0.161 (0.110)	–0.13 (0.203)	0.136 (0.243)	–0.181 (0.150)	–0.499[a] (0.0001)	0.274 (0.084)

Values are given as the standard coefficients determined by the multiple regression analysis and those of sex and body mass index are not shown because of no significant difference. *p* values given in parentheses. [a]$p < 0.05$.

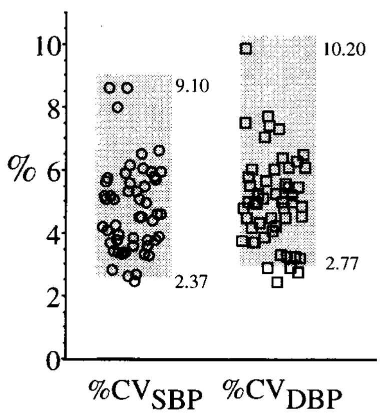

Figure 5. Coefficient of variation of beat-to-beat systolic (%CV_{SBP}(o)) and diastolic (%CV_{DBP}(□)) blood pressure in the supine position in Type 1 DM patients. The shaded area shows the normal limit with the upper and the lower values (mean ± 2 SD)

consistent with the findings by Aman *et al*.[6] However, Ringel *et al*. found decreased heart rate variation and the E – I difference during deep breathing in Type 1 DM patients compared to controls.[3] Weston *et al*.[29] found marked reductions in baroreflex sensitivity calculated from the Valsalva manoeuvre in subjects with diabetes, which were not detected by conventional tests. This apparent discrepancy may be due to metabolic control; the range of HbA_{1c} was wider in their patients than in ours with better metabolic control (Table 1).

Heart rate fluctuation in the supine position (%$CVRR_{UB}$) has often been used as an indicator of cardiac autonomic activity and reported to decrease in diabetic patients. Recent studies in Type 1 DM patients using power spectral analysis showed that decreased heart rate variation reflects reduction in the power density of the HF band in addition to the LF band with an increase of the LF/HF ratio,[30,31] indicating impairment of cardiac vagal tone. In keeping with these findings, CCVHF and CCVLF or R–R intervals tended to be low in our patients, but no patient was out of the normal range. Among various vagal function tests mentioned above CCVHF might be the best parameters, but is not as sensitive as blood pressure fluctuation.

It is difficult to explain that Type 1 DM patients had higher BP reponses to active standing at OS and after 7 min than controls. Orthostatic hypotension is a severe complication in the later stage of diabetes mellitus, but there is no report of higher blood pressure responses to postural change. Our Type 1 DM patients showed a square pattern during the Valsalva manoeuvre more commonly than controls, suggesting that patients with Type 1 DM might have higher plasma volume[32] because insulin depletion causes an increase in plasma volume.[33]

In conclusion, 7 diabetic children (12 %) had at least one abnormal result in the autonomic function tests; 5 in blood pressure fluctuation, 1 in deep breathing, and 1 in the Valsalva manoeuvre. Our data suggest that, in Type 1 DM patients aged between 6 and 22 years, the sympathetic nervous system of the peripheral vasculature is more likely to be involved than the cardiac autonomic nervous system. The Valsalva manoeuvre was not a

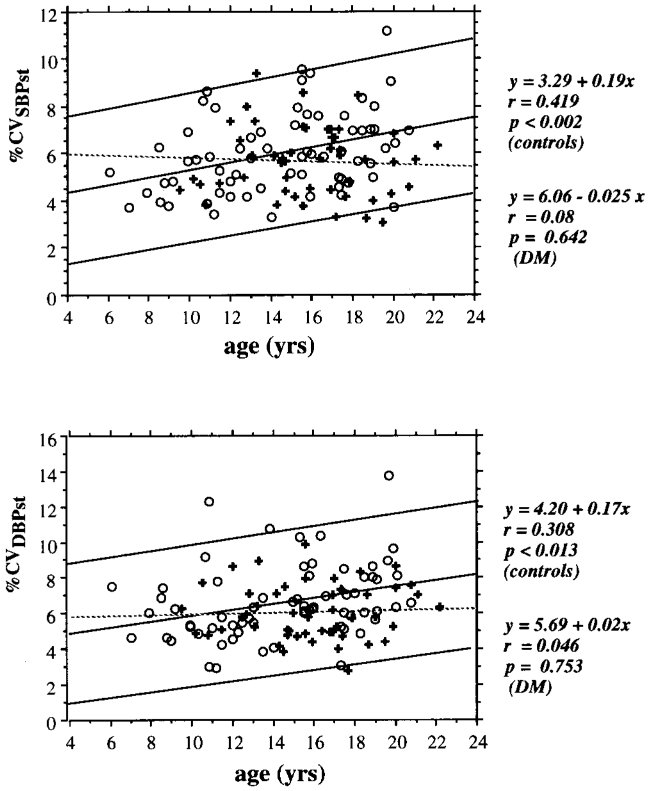

Figure 6. Plots of the coefficient of variation of beat-to-beat systolic (%CV_{SBPst}, upper panel) and diastolic blood pressure (%CV_{DBPst}, lower panel) during 4–7 min of standing in relation to age in patients with Type 1 DM (+) and in healthy controls (o). The regression line of patients (dashed line) and controls (straight line) with the upper and lower normal limit determined by prediction interval are shown

useable test for some children and the deep breathing test showed a lack of sensitivity. When autonomic function tests are performed in children, age-related changes and low reproducibility should be kept in mind. We found no evidence to support the use of power spectral analysis of heart rate variation in place of conventional autonomic function tests in children. Among various autonomic tests, beat-to-beat blood pressure fluctuation was most sensitive and reliable. This is a simple method and requires only a non-invasive continuous blood pressure monitor such as Finapres in addition to suitable software.

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